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MORPHOLOGICAL ORGANIZATION OF CRUSTACEAN PIGMENTARY EFFECTORS

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Abstract

The basic morphological organization of pigmentary effectors from the epidermis and internal organs of the freshwater palaemonid shrimps *Macrobrachium olfersii* and *Macrobrachium heterochirus*, the marine shrimps *Palaemon affinis* and *Palaemon northropi*, and the fiddler crab *Uca rapax*, was investigated by light and transmission electron microscopy. All pigmentary effectors (chromatosomes) examined comprise groups of 2–12 tightly bound, uninucleate cells (chromatophores), each possessing one or two cell extensions. Constituent chromatophores contained either a single or several pigment granule types of one or different colors. Desmosomes were noted between the membranes of adjacent chromatophores, although a limiting basement membrane was only present surrounding chromatosomes from the internal organs. Multinucleate or syncytial chromatophores were not encountered. Terminology currently used to describe crustacean pigmentary units is discussed in relation to the different levels of organization revealed.

Introduction

Although crustacean chromatophores have long been the subject of physiological investigations (see Fingerman, 1963, 1965, 1969, 1970; Fingerman *et al.*, 1975; Lambert and Fingerman, 1978, 1979, for reviews and references) and, more recently, of ultrastructural studies (Elofsson and Kauri, 1971; Chassard-Bouchaud and Hubert, 1971a, b; Green and Neff, 1972; Green, 1973; Robison and Charlton, 1973; McNamara, 1979, 1980, 1981a), the basic morphology of these pigmentary effectors is still unclear and the terminology used to describe them needs revision.

Pouchet (1876) considered each crustacean chromatophore to be a single cell containing a single pigment. However, Keeble and Gamble (1904, p. 304) stated of *Macromysis flexuosa* that "The mature chromatophores are not single cells but are complex organs." Of decapod crustacean chromatophores, these authors said "Several pigments may occur in the centre of a single chromatophore, and the evidence goes to show that each pigment occupies one or more 'cells' and the processes of these cells, and that the chromatophores are clusters of such." Their general conclusion was that "The chromatophores are polynuclear stellate masses of cytoplasm, the central part of which is bounded by a distinct membrane."

Ballowitz (1914) proposed the term *chromatosome* to describe pigmentary effectors comprising groups of closely united, unicellular chromatophores. However, this concept has since been modified, for example, by Barrington (1964, p. 300): "Among invertebrates chromatophores are best known in Crustacea where they may form syncytial complexes called chromatosomes," and by Nicol (1967, p. 510); "Adaptive colour responses are a notable feature of the behaviour of many deca-

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pods. The chromatophores concerned in these changes are multinucleate or syncytial structures lying in the skin, or sometimes at deeper levels."

Recent ultrastructural studies (Elofsson and Kauri, 1971; Hubert and Chassard-Bouchaud, 1976; McNamara, 1976, 1981b) confirmed Ballowitz' (1914) proposition that pigmentary effectors may be multicellular. Thus, chromatosomes contain separate chromatophores of similar or different colors, each color being represented by several cells. A basement membrane limiting the chromatosome has been described (Elofsson and Kauri, 1971).

To ascertain to what extent crustacean pigmentary effectors, including those exhibiting only a single color, are multicellular entities, the present study examines the basic morphological organization of the chromatic units of a variety of crustaceans including marine and freshwater palaemonid shrimps and a marine brachyuran crab. A suitable terminology is proposed to describe these effectors at all levels of organization.

Materials and Methods

Specimens of the freshwater palaemonid shrimps *Macrobrachium heterochirus* and *M. olfersii*, and the fiddler crab *Uca rapax*, were collected from small streams discharging into the São Sebastião Channel at or near the Guaecá Beach (23°49′18″S; 45°27′18″W) in the State of São Paulo, Brazil. The marine palaemonids *Palaemon northropi* and *P. affinis* were collected from intertidal rock pools near the Guaecá Beach, Brazil, and from the Kaikoura Peninsula, New Zealand (42°25′S; 173°42′E), respectively.

For light microscopic and ultrastructural examination, small pieces of brachiostegite, ventral nerve cord, and arthrodial membrane, containing chromatophores, were dissected and prefixed in a 0.1 M, Na-cacodylate buffered, paraformaldehyde-glutaraldehyde (65 mM: 250 mM) solution also containing Ca (12 mM), K (5 mM) and Mg (3 mM) as chlorides, for 1.5 h at 4°C. The material was subsequently washed at 4°C for 15 min, in three changes of the above solution minus the paraformaldehyde-glutaraldehyde, postfixed in 0.1 M, Na-cacodylate buffered, 1% osmium tetroxide for 2 h at 4°C, dehydrated in a cold ethanol series, passed through propylene oxide, and embedded in either Araldite 6005 or Spurr's (1969) resin. Thick (0.3–0.5 μm) and thin (silver-gold) sections were cut with glass knives on either an LKB Bromma Ultratome III or a Porter-Blum Sorvall MT-2 ultramicrotome. Thick sections were stained on a hotplate with a solution comprising equal parts of 1% methylene and toluidine blue in 1% aqueous borax. They were then examined in Zeiss or Leitz Orthoplan photomicroscopes. Thin sections were stained with 2% aqueous uranyl acetate followed by Reynolds' (1963) lead citrate. They then were examined in Jeolco JEM 100B, Philips EM 301 or Zeiss EM9S-2 electron microscopes at accelerating voltages of 60 or 80 kV.

Measurements were taken directly from the micrographs and are given in the text as the mean value ± the standard error of the mean. The number of measurements used (n) is given in parentheses.

Results

All the pigmentary effectors examined, regardless of species or location, proved to be groups of intimately associated, uninucleate, pigment bearing cells. In the integumental epidermis of the freshwater shrimp *Macrobrachium olfersii* such cellular composites, or chromatosomes, attain great complexity (Fig. 1), comprising

up to 12 uninucleate, monochromatic cells. Each chromatophore has a roughly spherical cell body (Fig. 2) of 25.0 ± 1.4 μm diameter (n = 15), from which project one, or at most two, cell extensions. At their junction with the cell body, these extensions, when filled with pigment, measure about 5 μm diameter, tapering to 1–2 μm diameter in their most distal regions. The entire chromatosome measures

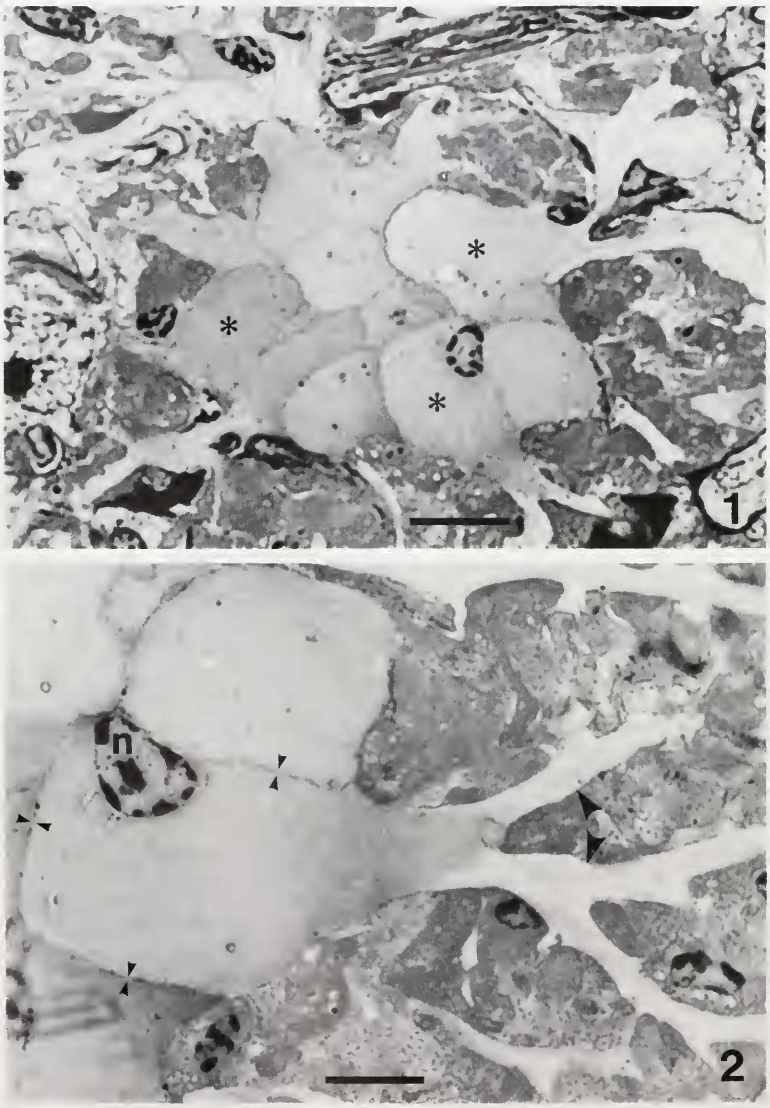


FIGURE 1. Light micrograph of longitudinal, thick section through red, epidermal chromatosome in *Macrobrachium olfersii*. Each component chromatophore (*) is distinct and uninucleate. Pigments are fully aggregated. Scale bar, 20 μm.

FIGURE 2. Light micrograph of longitudinal, thick section through a single, uninucleate chromatophore that was a part of red, epidermal chromatosome in *Macrobrachium olfersii*. Two cell extensions (arrows) derive from the cell body. Chromatophore boundaries (apposed arrows) are distinctly defined. Pigments are fully aggregated. Nucleus (n). Scale bar, 10 μm.

about 184.7 ± 12.4 μm diameter (n = 15), including the cell extensions. In these monochromatic, red chromatophores, the pigment granules are spherical to polymorphic, of 94.8 ± 3.7 nm diameter (n = 48), and lack a limiting membrane.

A very similar situation was revealed for the epidermal chromatosomes of the marine shrimp *Palaemon affinis* (Figs. 3, 4). The living chromatosomes of this shrimp contain at least three pigment colors. A peripherally located yellow pigment lies distally to a brown pigment, and large (approx. 1–2 μm diameter), iridescent blue, oblong granules are distributed throughout the chromatophore cell bodies and extensions. The yellow and brown pigments are not resolvable as granules at the light microscopic level in the living cells.

When seen in thin sections, the individual chromatophores composing these chromatosomes also contain at least three pigment granule types: One is a membrane-bounded polygon of 0.5–1.5 μm in the longest axis (the blue pigment crystal). An amembranous spherical granule of 140.0 ± 5.0 nm diameter (n = 150), encountered in the cell body, contains the brown pigment (Fig. 5). The yellow pigment, which appears as a similar, spherical granule of 80.0 ± 2.0 nm diameter (n = 250), is abundant in sections taken from the peripheral extremities of the chromatophore extensions. Both granule types can be seen mixed with the polygonal, membrane-limited pigment crystals. Such chromatophores are thus dichromatic, while the chromatosome is polychromatic.

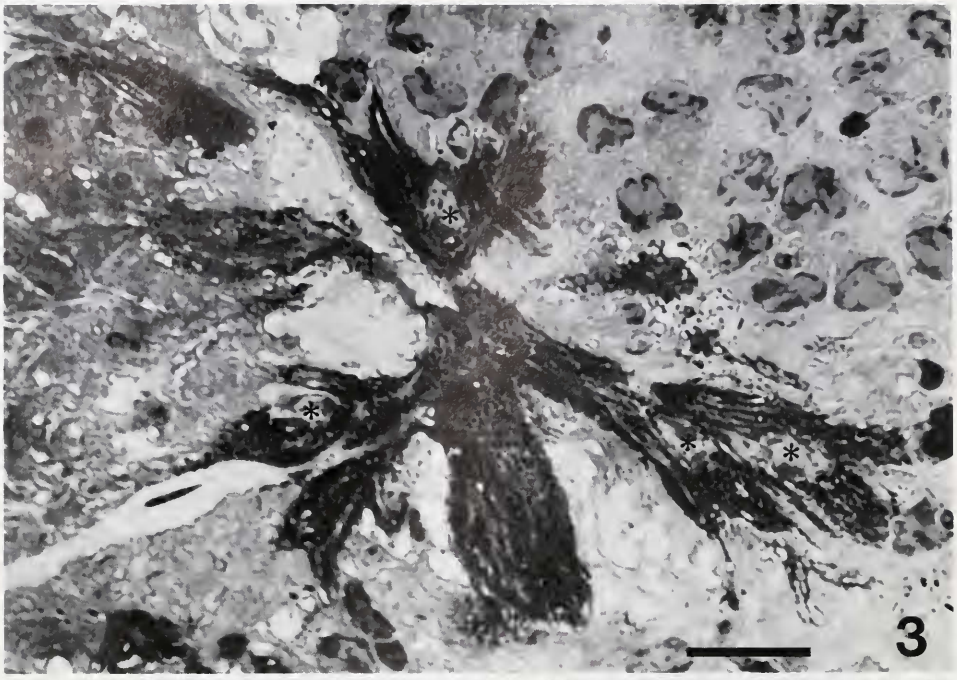
The brown chromatosomes enveloping the ventral nerve cord of the freshwater shrimp *Macrobrachium heterochirus*, although less complex than those of the epidermis, are small aggregates of 3–4 uninucleate chromatophores (Fig. 6). Component chromatophores, although exhibiting only a single color (brown), contain spherical, membrane-limited granules of 600.0 ± 50.0 nm diameter (n = 30) (Fig. 6), in addition to numerous spherical, amembranous granules of 71.4 ± 1.8 nm diameter (n = 30) (Fig. 7). The brown, ventral nerve cord chromatosomes of the marine shrimp *Palaemon northropi*, of similar basic organization, also contain two pigment granule types. One is a polygonal, membrane-limited granule of 400.0 ± 20.0 nm diameter (n = 24), which exhibits a paracrystalline substructure comprising alternating electron-dense and electron-lucent bands, repeating at 8.40 ± 0.13 nm intervals (Fig. 8). The other granule is spherical, of 51.1 ± 2.4 nm diameter (n = 20), and lacks a limiting membrane (Fig. 8).

In all the chromatosomes of the shrimp species examined, adjacent chromatophores are strongly bound to each other by well developed membrane junctions (Fig. 9). Only the ventral nerve cord chromatosomes are limited by a basement-membrane-like structure, probably composed of collagen fibres. Epidermal chromatosomes showed no evidence of a basement membrane, but exhibited more strongly developed areas of desmosomal contact.

The black epidermal chromatosomes of the brachyuran crab *Uca rapax* were the simplest encountered. These chromatosomes comprise only 2–3 chromatophores, each uninucleate (Fig. 10), containing a single type of highly electron dense, membrane-limited spherical pigment granule of 300.0 ± 10.0 nm diameter (n = 29). Small and infrequent membrane junctions bind the plasmalemmae of adjacent chromatophores within the chromatosome. A basement membrane was not noted.

Discussion

Morphological data from the present study clearly reveal that pigmentary effectors from the epidermis and internal organs of both natant and brachyuran decapods are closely bound groups of several uninucleate chromatophores. Single



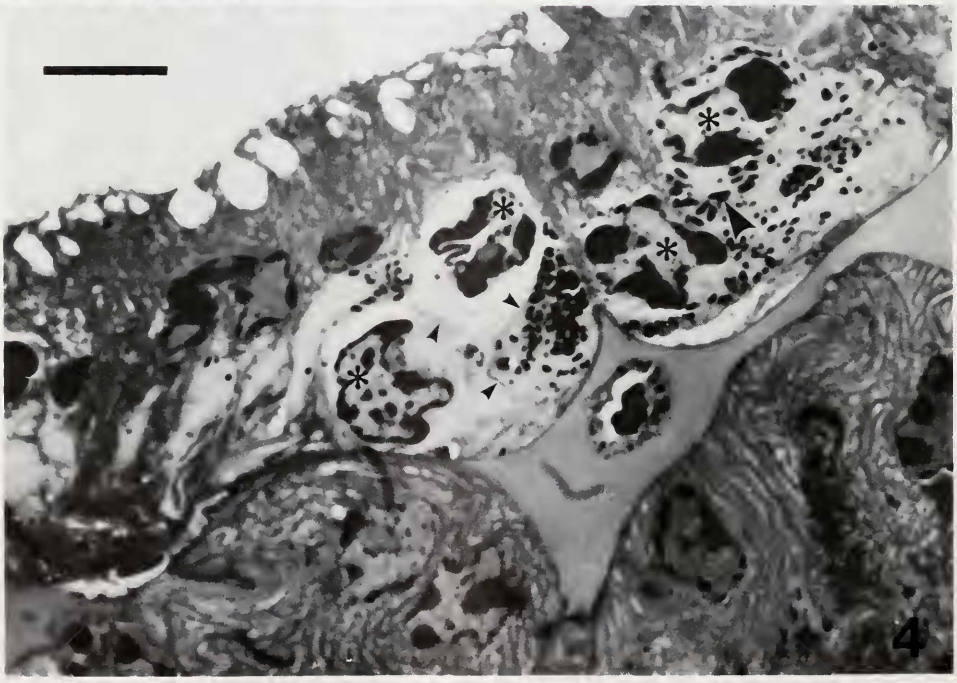


FIGURE 3. Light micrograph of longitudinal thick section through a polychromatic epidermal chromatosome in *Palaemon affinis*. Several chromatophores are present, each uninucleate. Pigments are fully dispersed. Nucleus (*). Scale bar, 20 μm.

FIGURE 4. Light micrograph of transverse section through polychromatic, epidermal chromatosome in *Palaemon affinis*. Several uninucleate chromatophores, separated by distinct boundaries (small arrows) are discernable. The darkly staining polyhedrons (large arrow) are blue pigment crystals; the lightly staining areas are brown pigment (*cf.*, Fig. 5). Pigments fully dispersed. Nucleus (*). Scale bar, 10 μm.

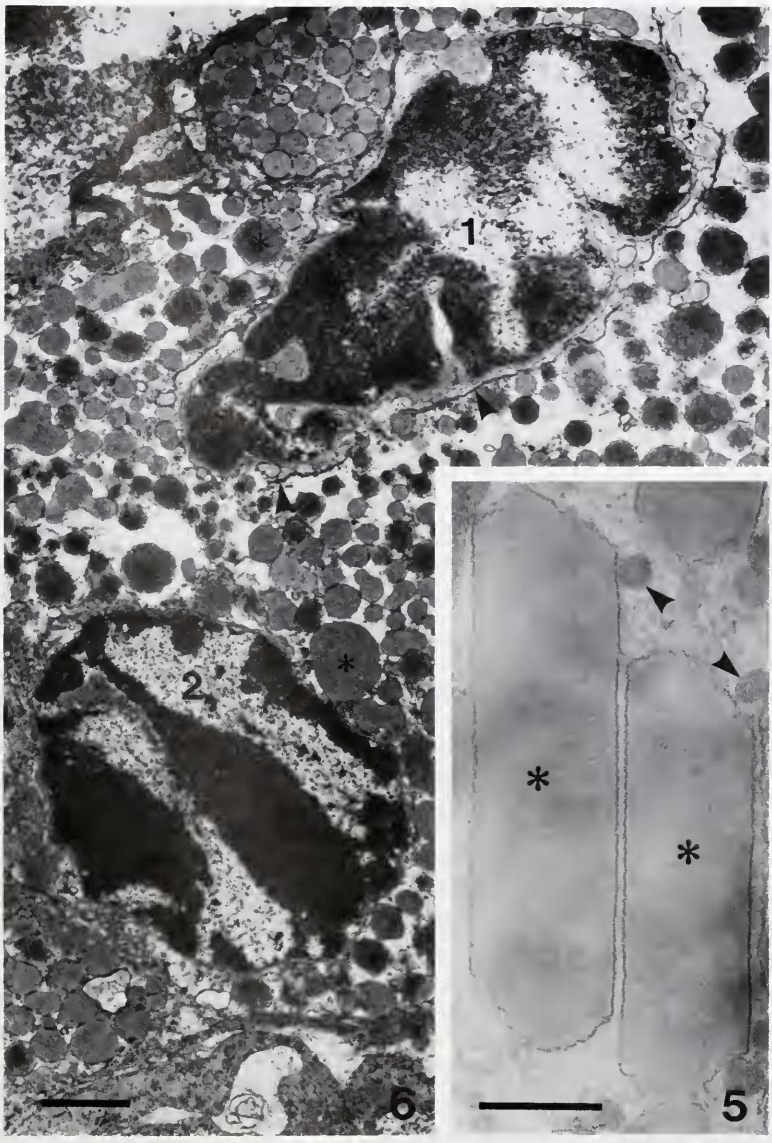
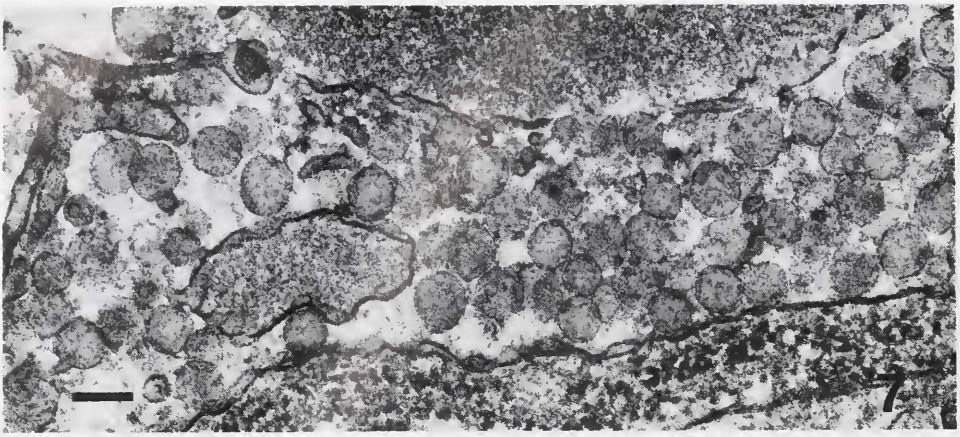


FIGURE 5. Membrane-bounded polyhedral pigment crystals (*) and amembranous spherical pigment granules (arrows) in dichromatic epidermal chromatophore from *Palaemon affinis*. Scale bar, 0.4 μm.

FIGURE 6. Transverse section through brown chromatosome from *Macrobrachium heterochirus* ventral nerve cord, revealing multicellular nature. Nuclei (1, 2) are separated by two closely apposed plasmalemmae (arrows). Note large, membrane-bounded spherical pigment granules (*). Scale bar, 1.5 μm.

chromatophores are rare. Each component chromatophore may contain a single type of pigment granule, *e.g.*, the monochromatic red epidermal chromatophores of *Macrobrachium olfersii* and the monochromatic black epidermal chromatophores of *Uca rapax* (*cf.* Green and Neff, 1972), or may contain several pigments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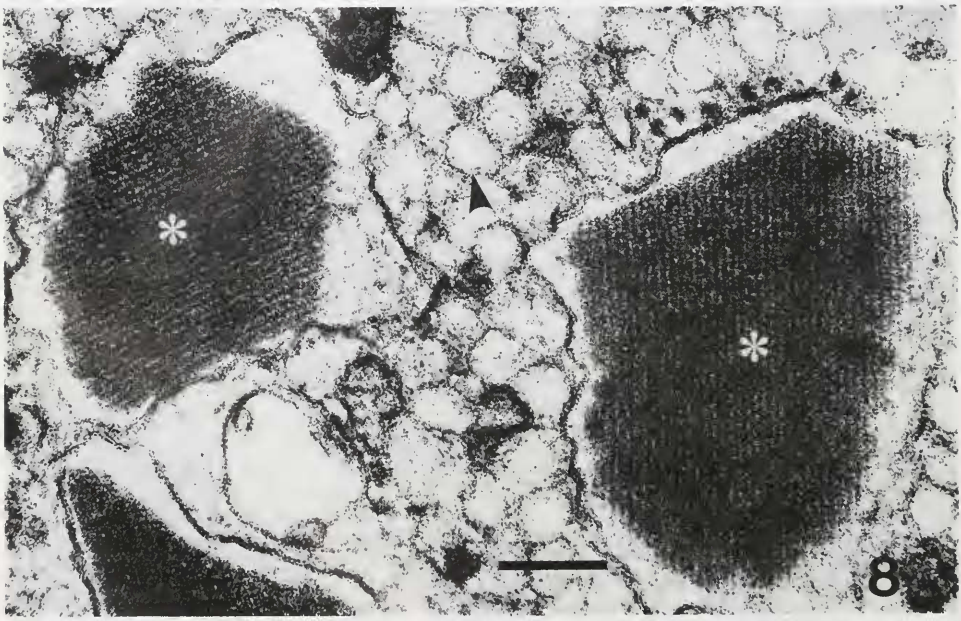
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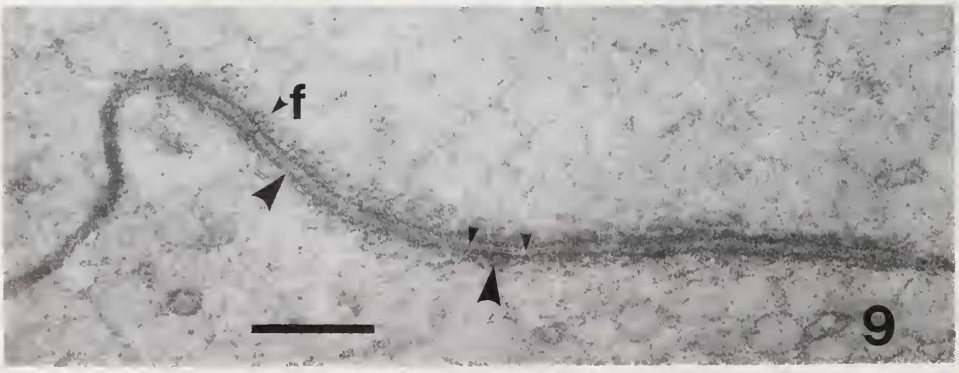
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of very different morphology and color, *e.g.*, the dichromatic, epidermal chromatophores of *Palaemon affinis*. Robison and Charlton (1973) found up to four different pigment granule types within a single ovarian chromatophore of *Palaemonetes vulgaris*, while Hubert and Chassard-Bouchaud (1976) reported two morphologically distinct granules in red, epidermal chromatophores of *Palaemon serratus*. McNamara (1981a) described three pigment granule types from the hind gut chromatophores of *Palaemon affinis*. In the above examples, the different pigment granules were the source of different pigment colors. However, in the brown chromatosomes of the ventral nerve cord of *Macrobrachium heterochirus* (and other *Macrobrachium* species; McNamara, 1981b) only a single color is visible even though two morphologically distinct pigment granules are present in the component chromatophores. Such chromatophores are, strictly, monochromatic. Thus, considered at the level of the smallest functional unit, the individual chromatophore may contain pigment of a single color (regardless of the number of granule types present), being therefore monochromatic, or may contain several pigments of different colors and morphologies, being di- or polychromatic.

Chromatosomes likewise differ considerably, particularly in the number of constituent chromatophores. They are probably simplest in the brachyuran crabs, comprising 2–3 cells, and most complex in the palaemonid shrimps, where up to 15 cells may be encountered. All the chromatophores composing the chromatosome may contain the same pigment (monochromatic chromatosome), *e.g.*, red, epidermal chromatosomes of *Macrobrachium olfersii* and black, epidermal chromatosomes of *Uca rapax*; or they may contain different pigments, each chromatophore bearing pigment of a single color (Elofsson and Kauri, 1971) (polychromatic chromatosome). The most complex situation, thus far noted only in palaemonid shrimps, *e.g., Palaemon affinis*, is that in which di- or polychromatic chromatophores containing, separately, brown and blue, yellow and blue, and red and blue pigments, are united to form polychromatic chromatosomes.

Neither chromatosomes nor chromatophores are multinucleate or syncytial; this idea probably arose as a misinterpretation of light microscopical studies using liver material. Parker (1948, pp. 54–55) discussed this aspect. His proposed terminology is satisfactory in light of the present study, although chromatophores proper are now known to contain more than one kind and color of pigment. Brown (1973, p. 917) stated that "the several pigments within a single chromatophore may show a considerable degree of independence of one another." This observation now may well be reinterpreted as reflecting the independence of pigment translocation within a single chromatophore type comprising the chromatosome. However, although the smallest functional unit of the crustacean pigmentary effector is the chromatophore, the chromatosome probably forms the basis of any chromatic arrangement.

The presence of a basment-membrane-like structure limiting the chromatosome (Elofsson and Kauri, 1971) was only verified for the chromatosomes situated on

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FIGURE 7. Amembranous spherical pigment granules from brown ventral-nerve-cord chromatophore in *Macrobrachium heterochirus*. Scale bar, 0.1 μm.

FIGURE 8. Paracrystalline membrane-limited pigment granules (*) and spherical amembranous pigment granules (arrow) in brown ventral-nerve-cord chromatophore of *Palaemon northropi*. Scale bar, 0.1 μm.

FIGURE 9. Desmosome (large arrows) formed between plasmalemmae of two adjacent chromatophores in red, epidermal chromatosome of *Macrobrachium olfersii*. Note fine electron-dense line (arrows) between the plasmalemmae, and the fibrous material (f) below each membrane. Scale bar, 0.2 μm.

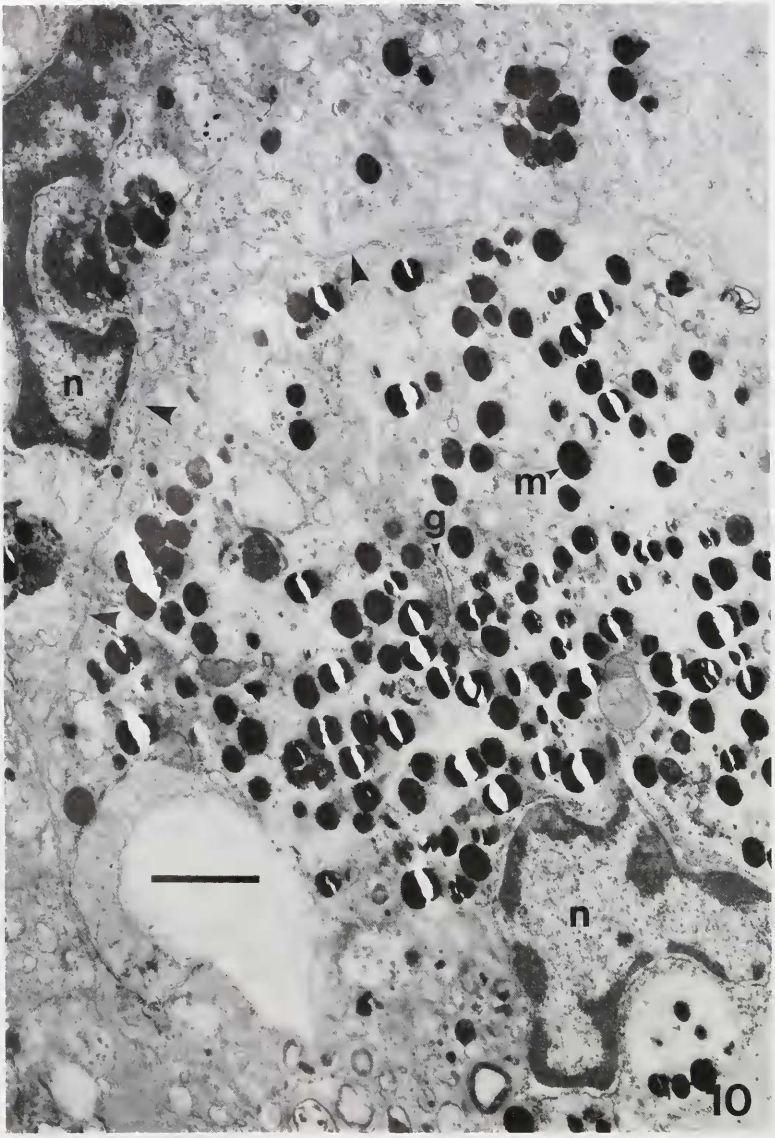

FIGURE 10. Longitudinal section through black, epidermal chromatosome in *Uca rapax*. Two clearly defined uninucleate chromatophores are discernable. Arrows indicate limiting membranes of each chromatophore. Granular endoplasmic reticulum (g), melanin granule (m), nucleus (n). Scale bar, 1 μm.

the internal organs, *e.g.*, ventral nerve cord and hind gut (McNamara, 1981a). Electron microscopical examination of epidermal chromatosomes in both shrimps and crab did not reveal such a structure. Desmosomes have not been previously demonstrated in crustacean chromatosomes.

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